REMARKS/ARGUMENTS

THE INVENTION.

As currently claimed, the invention now recites a method of discriminating between prostate cancer and BPH using mass spectroscopy to simultaneously detect multiple marker proteins below 10 kDa. Applicants believe that they were the first to recognize that such diagnostic discrimination was possible using multiple markers analyzed by mass spectroscopy [MS]. MS is particularly well suited for such analyses.

INTERVIEW SUMMARY

Pursuant to Rule 133, applicants acknowledge the interview of July 28 with Examiner Brandon Fetterolf. A proposed claim was faxed to the Examiner prior to the interview. During the interview the applicants discussed the prior art and their intent to present the claims set forth herein. No agreement was reached as to patentability.

STATUS OF CLAIMS

Claims 1-83 were pending. Claims 1-17 and 19-23 were examined and rejected. Claims 18 and 24-83 were restricted out and withdrawn. This amendment cancels claims 2-7, 9-11, 13-19 and 21-83. Claims 1, 8, 12, 20 and 84-88 are pending.

Claim 1 is amended by more expressly defining the steps. Support for the amendment are as follows:

The step of: **obtaining from a subject a sample containing a plurality of prostate related protein markers having apparent molecular weights below 10,000 Da,** finds support on page 2, lines 18-29, page 3 at lines 14-16 (reciting subjects) and page 26 lines 1-22 describing obtaining prostrate samples and at page 2, line 34 specifying 10 kDa as a preferred size.

The step of: **resolving by mass spectroscopy a test amount of the plurality of protein markers in the sample, the protein markers having an apparent molecular weight of less**

than 10,000 Da, finds support throughout the specification and expressly on page 3, line 25 reciting MS and in the examples where as depicted in Figure 3, this size range offers a particularly dramatic difference between cancer and BHP samples.

The step of: **comparing the test amount of the plurality of protein markers having apparent molecular weight of less than 10,000 Da with an amount of a plurality of protein markers having an apparent molecular weight of less than 10,000 Da from a control sample where the control sample originates from benign prostate hyperplasia patients,** finds support through out the specification where determination of difference are set forth by comparing and expressly at pages 26-28 and at page 27, lines 14, 20 and 31 where comparison with control language is used.

The last step of: **determining whether the test amount is a diagnostic amount consistent with a diagnosis of prostate cancer versus benign prostate hyperplasia,** finds support in the original claim 1 and in the specification at page 2, lines 18-24, page 26, line 8, page 27, and lines 22-23.

Newly added claims 84-88 find support as follows:

Claim 84 reciting protein markers adsorbed onto a probe comprising an adsorbent of a hydrophilic polymer finds support at page 17, lines 28-29.

Claim 85 reciting protein markers adsorbed onto a probe comprising metal binding groups finds support at page 19, line 35.

Claims 86-88 depending from claim 84 reciting various protein binding groups finds support at page 17, lines 21-23.

SPECIES ELECTION

Applicants affirm election of a species having an apparent molecular weight of 5753 Da.

INFORMATION DISCLOSURE STATEMENT

Applicants appreciate the Examiner considering the references cited in the International Search Report filed on March 25, 2002. A proper form has been enclosed in order to have the references appear printed on a resulting patent.

SPECIFICATION

The Examiner objected to the specification for two reasons. First, he indicated that the description of Figure 2 on page 5 was missing. Reconsideration or clarification is requested. Enclosed is a copy of page five of the published PCT application. The figure description for Figure 2 is present at the bottom of the page.

Second, a typographical error was noted at page 33, line 20. The spelling error has been corrected by amendment.

REJECTIONS

35 U.S.C. § 112 2nd ¶

The Examiner rejected original claims 1-17 and 19-23 as incomplete for failing to describe essential steps. As explained by the Examiner, he requested that the claims recite the correlation step of comparing cancer samples to BPH samples. As amended, independent claim 1 now recites these steps.

35 U.S.C. § 112, 1st ¶ - Description

The Examiner rejected the original claims because they read on the use of undescribed markers when only a representative group of markers were provided. Reconsideration in view of the amendments to the claims is requested.

To the extent that the Examiner may maintain the description rejection over amended claim 1, applicants note that two cases cited by the Examiner are distinguishable from the subject invention. In *Lilly*, the UC was claiming human insulin when it had only provided the world with rat insulin. In the *Rochester*, the invalidated claims read on treating a patient with **any** drug that inhibited enzyme A but not enzyme B. Depending on how you interpret the

decision, Rochester had not disclosed any examples of such compounds or perhaps a single example.

In both the *Lilly* and *Rochester* decisions, there was a clear failure to describe the invention and to enable it. In the subject invention, as amended, the claims recite a method of distinguishing between two disease states by evaluating and characterizing a plurality of small peptides/protein markers using MS. The beauty of the method is that you don't need to determine what the markers are exactly—you can, but it is not necessary to do so. The mass profile is all that is needed. It is the profile that is described. To the extent that the mass profile can be further characterized according to its components having different molecular weights, this is not needed to describe the invention as presently claimed because the specification clearly discloses that the inventor possessed the invention as claimed and thereby has placed the invention in the possession of the public. *Possession* is the gravamen of the description requirement (see also MPEP §2163 - 3.(a) .[1]

35 U.S.C. § 112, 1st ¶ - Enablement

Original claims 1-17 and 19-23 were further rejected as lacking enablement because not all markers distinguishing between prostate cancer and BPH were taught. As amended, the claims now recite a general MS procedure for distinguishing between the two disease states. The claim recites the step of "resolving by mass spectroscopy a test amount of the plurality of protein markers in the sample."

Thus, the claims do not rely on identification of specific markers, but use the power of MS to generate mass profiles to quantify a plurality of proteins by size in a rapid and cost-effective manner.

Having limited the claims to embrace the step of resolving a plurality of markers via MS and generating data based on molecular weight, there is no longer any need to evaluate

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[1] In re Edwards, 196 USPQ 465, at 467 (CCPA 1978).

To comply with the description requirement it is not necessary that the application describe the claimed invention in ipsis verbis, ... ; all that is required is that it reasonably convey to persons skilled in the art that, as of the filing date thereof, the inventor had possession of the subject matter later claimed by him.

the data to determine what specific markers are present in the sample. You could do this, but it is not necessary to practice the invention.

More specifically, the enablement requirement demands that the patent applicant teach those of skill how to practice the invention as claimed without undue experimentation. In view of the amendments to the claims, applicants have focused their claims and their invention to a scope that is clearly taught by example and detailed teachings. They teach that a MS generated mass profile generally capturing multiple proteins can, by virtue of the quantity of the sized proteins, be a useful tool to distinguish between prostate cancer and BPH. In addition, there are detailed examples and general teachings of how to accomplish this in a variety of ways. Nothing more is required under the law of enablement. In view of the claim amendments, applicants submit the claimed invention is now fully enabled.

35 U.S.C. § 102

The Examiner rejected original claims 1, 6, 8 and 10 as anticipated by Zetter *et al.*. Zetter teaches the use of Thymosin B15 to differentiate between prostate cancer and BPH. Thymosin B15 inherently has a molecular weight of less than 27 kDa.

Applicants have amended claim 1 to avoid the novelty rejection. Rejections under §102 require an examiner to identify a single reference disclosing all of the salient features of a claim. By reciting a plurality of markers less than 10,000 Da and the use of MS to resolve the plurality of markers, the Zetter reference can no longer be cited as a novelty bar against the rejected claims. Reconsideration of the rejection in view of the amendments is requested.

35 U.S.C. § 103.

The Examiner rejected original claims 1, 6-8 and 10-12 as obvious over Zetter in view of Hutchens. Hutchens discloses the use of MS to discriminate between proteins after selective washing and eluting of samples on an MS probe. The Examiner creates his *prima facie* case of obviousness by urging that one of skill would be motivated to use the MS methods taught by Hutchens to identify the marker of Zetter.

Reconsideration of the rejection in view of amendments is requested. The amended claims now recite the use of MS to resolve a plurality of small molecular weight markers to distinguish between prostate cancer and BPH. The prior art would not have anticipated that MS could be used in such a powerful manner.

To some extent, the combination of Zetter and Hutchens might lead one of skill to detect Thymosin B15 via MS, but there is little motivation to capture a plurality of proteins of less than 10 kDa to distinguish between the two disease states. More specifically, applicants submit that the combination of references teaches or motivates away from capturing a plurality of proteins while motivating those of skill towards specific protein capture methods - MS based or otherwise.

In fact when one looks at the "hypothetical" invention underlying any obviousness rejection as applied to the amended claims, the invention fails in the very way articulated above. The Examiner is asked to take note that Thymosin Beta 15 is calculated to have a mw of 5.3 kDa (see Exhibit 1, page 257, second column). In contrast, Figure 3 of the subject application does not reveal any peak at 5.3 kDa. There are two possible reasons for this—either the MS probe does not bind Thymosin Beta 15, or the concentration of this protein is below the dynamic range of the MS detection.

Under either scenario, one of skill attempting to combine Zetter and Hutchens would be discouraged from looking at general protein capture technology and focus on increasing selectivity and sensitivity to detect thymosin beta 15. This could be accomplished perhaps by thymosin-specific binding agents in combination with MS and/or more likely with alternative non-MS technology as suggested by Zetter. Either way, it is clear that the reality of the hypothetical combination of Zetter and Hutchens would clearly motivate those of skill **away** from the invention as claimed, i.e., resolution of a **plurality** of proteins below 10 kDa via MS.

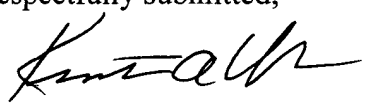
Reconsideration of the rejection in view of the amendments to the pending claims and the above argument is respectfully requested.

CONCLUSION

In view of the foregoing, Applicants believe that all the Examiner's concerns have been addressed and that all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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Endocrine-Related Cancer (1998) 5 253-270

The role of stroma in prostatic carcinogenesis

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Abstract

Most human prostate tumors are adenocarcinomas which arise from the epithelial cells that line the glands and ducts of the prostate. Consequently, the malignant epithelial cell, or more specifically genetic damage suffered by that malignant epithelial cell, has been the major focus of prostate cancer research to date. There is, however, increasing evidence to suggest that alterations in the stromal microenvironment associated with a malignant epithelium may be necessary for progression of carcinogenesis.

We have recently hypothesized that interactions between the stroma and epithelium become altered as a result of genetic damage to the prostatic epithelial cell. During prostatic carcinogenesis, this abnormal signaling may lead to changes in both the prostatic epithelium and smooth muscle with concomitant loss of growth control. In this way, both a malignant epithelium and an abnormal or 'tumor stroma' evolve.

The purpose of this article is to describe interactions between the stroma and epithelium of the normal prostate, and then to summarize evidence suggesting that stromal cells derived from benign versus malignant sources may exert differential effects on epithelial cell growth and differentiation.

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Introduction

Cancers of the prostate, lung and colon are among the most common malignancies diagnosed in men living in the Western world. In fact, recent estimates have placed prostate cancer as the most commonly diagnosed malignancy, and the second leading cause of cancer-related death among men in the United States (Landis *et al.* 1998). Prostate cancer is comparatively less common in other parts of the world, where the incidence and/or mortality rates of other tumors, such as those that arise from the mouth, pharynx and stomach exceed those of prostate cancer.

Prostate cancer is unique among carcinomas in that it is a slow growing malignancy that is diagnosed almost exclusively in men over 50 years of age. Consequently, when determining survival benefits associated with any of the currently available forms of local treatment, at least ten to fifteen years of follow-up is required before a true survival benefit can be confirmed. Thus, for an individual patient with competing co-morbidities, overall life expectancy must be taken into consideration before definitive local treatment can be recommended. It is for this reason that some patients may require no treatment whatsoever. This group may include patients who are elderly with significant co-morbid conditions as well as those patients with low grade, low volume disease. In this regard, 'watchful waiting' has become a treatment option that is unique to prostate cancer (Johansson *et al.* 1992, Chodak *et al.* 1994).

For most men diagnosed with prostate cancer, however, there is a high risk of disease progression if no treatment is delivered. In these patients, the benefits derived from local treatment with respect to disease-free and overall survival must be balanced against potential side-effects associated with such treatment. For patients with favorable disease characteristics, such as a low pre-treatment serum prostate specific antigen (PSA), a low clinical stage, and a well or moderately differentiated tumor, disease-free and overall survival five years following treatment is excellent utilizing either radical prostatectomy or radiotherapy as definitive local treatment. However, these results have not been as encouraging for patients with higher risk disease

Endocrine-Related Cancer (1998) 5 253-270 *Printed in Great Britain*
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EXHIBIT 1

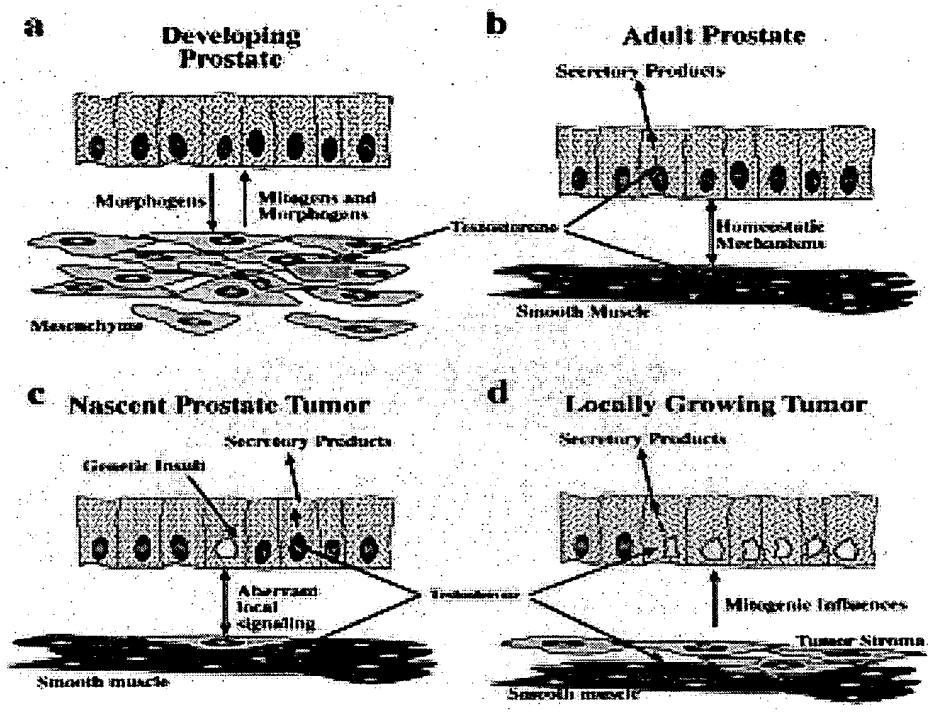

Figure 1 Schematic representation of interactions between the prostatic stroma and prostatic epithelium during normal development, adulthood and carcinogenesis. (a) Testosterone acts through androgen receptors (AR) in the urogenital sinus mesenchyme to induce epithelial development and differentiation. In a reciprocal fashion, epithelium signals to mesenchyme to induce smooth muscle differentiation. (b) In the growth-quiescent adult prostate, testosterone acts through both smooth muscle and epithelial AR. Androgens act through prostatic smooth muscle AR to maintain prostatic epithelium in a fully differentiated, growth-quiescent state and through epithelial AR to stimulate secretory function of the fully differentiated epithelium. It is hypothesized that the epithelium also acts to maintain smooth muscle differentiation through paracrine acting factors. (c) Prostatic carcinogenesis appears to be initiated by genetic insult to the epithelium. This leads to a change in epithelial phenotype and aberrant local signaling between the epithelium and smooth muscle. (d) The aberrant local signaling described in (c) leads to phenotypic changes in both the stromal and epithelial compartments of the tumor. The stroma becomes a fibroblastic 'tumor stroma' which promotes epithelial mitogenesis. This would predictably lead to increased epithelial proliferation, migration and, ultimately, an invasive epithelial phenotype.

characteristics (Catalona & Smith 1994, Walsh *et al.* 1994, Zietman *et al.* 1994, Hanks *et al.* 1995, Zagars & Pollack 1995). These results, in combination with the potential side-effects that have been associated with both surgery and conventional external beam radiotherapy (including urinary incontinence, rectal complaints and impotence), have led to several newer treatment options aimed at providing better results with fewer side-effects. However, any benefit of these newer treatment modalities with respect to disease-free survival or improved quality of life remains unproven.

Our laboratory has begun to examine some of the unique biological characteristics associated with prostate cancer with the ultimate goal of formulating novel, non-invasive treatmentment strategies. More than 95% of human prostatic cancers are adenocarcinomas which arise from the epithelial cells that line the glands and ducts of the prostate (Stamey & McNeal 1992). Consequently, most research to date on prostate cancer has examined changes occurring in the prostatic epithelial cell as it progresses from a normal to a frankly malignant carcinoma cell. There is, however, a growing body of evidence to suggest that, as a carcinoma evolves, changes also occur in the stromal compartment associated with the tumor. In many instances these changes may serve to enhance the invasive and/or malignant potential of the nascent epithelial tumor. With this in mind, we have hypothesized that epigenetic influences originating from stromal cells in the immediate vicinity of a prostatic tumor may be critical in determining whether a particular tumor

assumes a slowly growing or an invasive phenotype (Cunha *et al.* 1996, Hayward *et al.* 1996*b*, 1997*b*). It is possible that, following genetic alteration to the prostatic epithelium, signaling from the epithelium to the surrounding smooth muscle becomes aberrant. This may result in stromal dedifferentiation towards a fibroblastic phenotype. One of the consequences of such a transformation may be that the local microenvironment changes from promoting epithelial homeostasis to promoting epithelial mitogenesis. These changes would be predicted to lead to increased epithelial prolif-eration, migration and, ultimately, could enhance the in-vasive potential of the genetically altered epithelial cell (Figs 1 and 2).

The purpose of this article will be (1) to describe the stroma of the normal (non-malignant) prostate, (2) to summarize genetic changes that are known to occur in the prostatic epithelium during carcinogenesis, (3) to present evidence suggesting that stroma derived from non-malignant sources may be able to alter the malignant phenotype of prostatic carcinoma cells, (4) to summarize the evidence in support of a 'tumor stroma', and (5) to summarize data describing the role of tumor stroma in prostatic carcinogenesis.

The stroma of the normal prostate

The human prostate is composed of two compartments: (1) an epithelial compartment, which includes the exocrine glands with their associated ductal structures, and (2) a surrounding connective tissue stroma. The stroma of the human prostate consists of a number of different cell types. The most abundant cell type in this compartment is the smooth muscle cell, which is derived

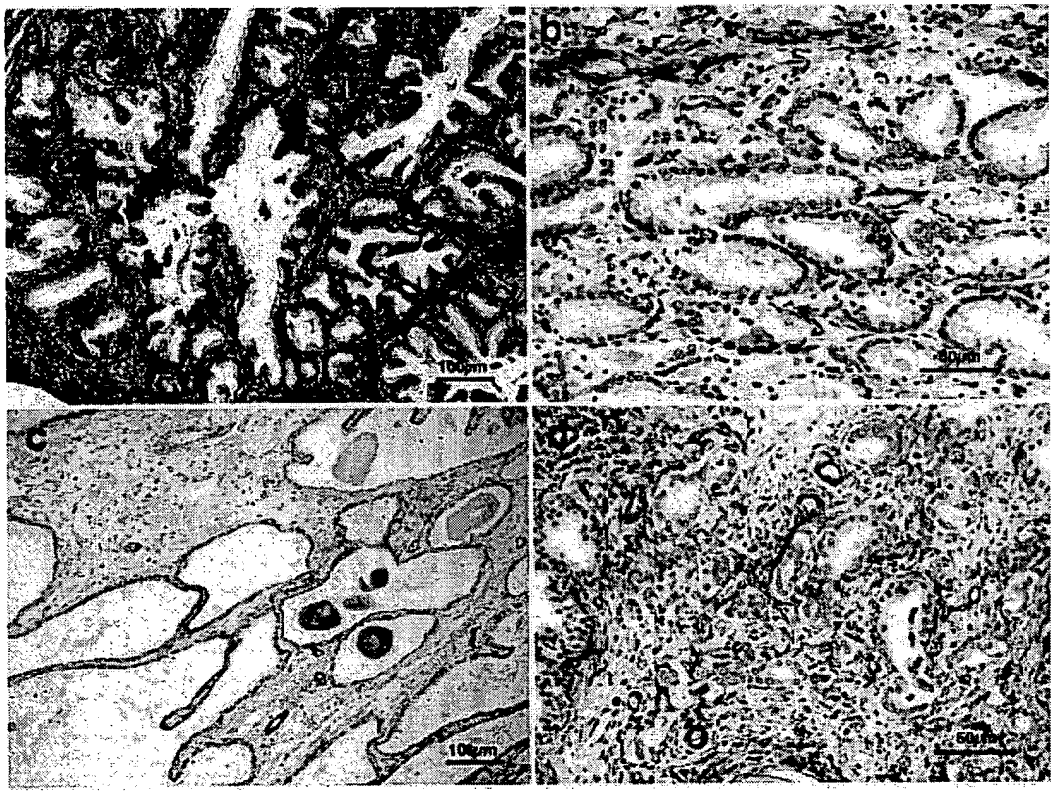

Figure 2 Immunohistochemical expression of smooth muscle α-actin and vimentin in the stroma of normal prostate and prostate cancer. (a) Smooth muscle α-actin expression in normal prostate. Note that the stroma is comprised predominantly of smooth muscle. (b) Smooth muscle α-actin expression in the stroma associated with prostate cancer. Note the paucity of immunostaining in this high power field. (c) Vimentin expression in normal prostate. Other than in blood vessels (which serve as positive internal controls), there is virtually no vimentin staining in the stroma of the normal prostate. (d) Vimentin expression in prostate cancer. Note the increased expression of this fibroblastic marker in the stroma associated with prostate cancer compared with the stroma of the normal prostate. The findings summarized in panels a-d suggest that the predominantly smooth muscle stroma of the normal prostate is altered in prostatic carcinogenesis to include vimentin-positive fibroblastic cells.

from the mesenchyme of the embryonic urogenital sinus (UGS). Other cell types located in the stroma of the normal adult prostate include fibroblasts, nerves, endothelial cells and vascular smooth muscle cells. In addition to being the most abundant stromal cell type, the smooth muscle cell appears to be the most important cell type with respect to prostatic development and maintenance of homeostasis. In this regard, changes in the smooth muscle cell may be important in the evolution of prostatic carcinogenesis (see below).

Tissue recombination experiments utilizing the androgen insensitive testicular feminized (tfm) mouse have established that an androgen responsive stroma is necessary for the development of normal prostatic epithelial architecture in the rodent (Cunha *et al.* 1987). In adulthood, prostatic smooth muscle cells, which are known to express androgen receptors (AR), interact with epithelial cells and under androgenic conditions maintain the epithelium in a fully differentiated, growth-quiescent state (Cunha *et al.* 1996). This occurs both in the presence and in the absence of epithelial AR expression which emphasizes the paracrine nature of smooth muscle-epithelial interactions. Human prostatic smooth muscle cells, which also express AR, are believed to play a similar role in maintaining prostatic homeostasis. However, definitive experiments to confirm this role for human prostatic smooth muscle cells are not possible given our inability to access AR-deficient human fetal urogenital sinuses.

Fibroblasts, which make up a large proportion of the stroma of the rodent prostate, are found sporadically in the normal human prostate. It has been suggested that these cells are important in mediating epithelial proliferation in the rodent prostate (Nemeth & Lee 1996). Their function in the human prostate remains unclear; they may serve as a scaffolding to keep smooth muscle bundles together, or they may play an active role in organ homeostasis.

Prostatic development occurs as a direct result of androgenic stimulation of the fetal UGS. Analysis of tissue recombinants composed of AR-positive wild type urogenital sinus mesenchyme (UGM) plus AR-negative tfm epithelium indicates that androgens act through AR in the mesenchymal cells of the UGS to stimulate epithelial proliferation, ductal branching morphogenesis, and columnar cytodifferentiation. In rats and mice, prostatic tissue can develop from the UGS derived from either a male or a female embryo if appropriately stimulated by androgens at critical developmental stages. In laboratory rodents the initial phases of prostatic budding require exposure to androgens prenatally. In contrast, ductal branching morphogenesis, canalization and epithelial cytodifferentiation all require postnatal androgenic stimulation. These early developmental events occur between birth and the onset of puberty, during which time circulating androgen levels are very low. These observations indicate that the developing prostate is extremely sensitive to low levels of circulating androgen (Donjacour & Cunha 1988).

Interactions between stroma and epithelium appear to be reciprocal in nature. Not only does the developing stroma induce epithelial development, but the developing epithelium also induces primitive mesenchymal cells to differentiate into smooth muscle (Cunha *et al.* 1992*b*). Urogenital sinus mesenchyme grown in the absence of epithelium will not form smooth muscle. Thus, the development of prostatic smooth muscle *in vivo* requires the presence of both epithelium and appropriate androgenic stimulation. This ability of epithelium to induce the formation of visceral smooth muscle is not restricted to the prostate, but it appears to be a common feature of many organs including the gut, uterus and bladder (Haffen *et al.* 1982, Cunha *et al.* 1989, Baskin *et al.* 1996).

The differentiation of prostatic smooth muscle occurs in an orderly manner with the sequential expression of a number of characteristic markers, including vimentin, actin, myosin, desmin and vinculin. The adult prostate, in which the stroma contains fully differentiated smooth muscle cells, is essentially growth-quiescent and maintains very low and balanced levels of cellular proliferation and cell death. It should be emphasized that this growth-quiescent, homeostatic state exists in the presence of high levels of circulating androgens. In the adult rodent, androgens act directly on the prostatic smooth muscle cells to maintain this fully differentiated growth-quiescent state (Hayward *et al.* 1996*b*). We have postulated that androgens act in a similar fashion in the adult human prostate to maintain growth-quiescence (Hayward *et al.* 1996*b*). Evidence supporting this hypothesis is as follows. After castration, the well recognized rapid regression of prostatic epithelium is associated with an ordered loss of expression of the various smooth muscle differentiation markers. This appears to reflect a 'dedifferentiation' of the smooth muscle cells. The loss of smooth muscle markers following castration occurs in the order opposite to that to which these markers were expressed during normal development (Hayward *et al.* 1996*a*). In its final form, the prostatic stroma of a long-term castrated animal reverts to a stroma which contains fibroblasts or mesenchymal cells that coexpress AR and vimentin. Very little expression of the characteristic smooth muscle markers is evident in the fully regressed prostate.

If exogenous androgens are subsequently administered to a long-term castrated animal, prostatic tissue will respond in a highly coordinated manner, with the differentiation of both stromal smooth muscle (again expressing its characteristic markers) and a secretory

epithelium (Bruchovsky *et al.* 1975). Thus, the relatively undifferentiated fibroblastic cells in the prostatic stroma of a castrated animal can respond to androgens by inducing epithelial proliferation and columnar cyto-differentiation while they revert back to highly differen-tiated smooth muscle cells.

In summary, low levels of circulating androgens act upon the mesenchymal cells of the developing prostate to induce prostatic epithelial proliferation and differentiation. In contrast, high circulating levels of androgen in the adult act through the prostatic smooth muscle to maintain a fully differentiated, growth-quiescent epithelium. Proliferative effects of stroma on epithelium are mediated through the stromal AR, while the epithelial AR appears to be required only for the expression of prostatic secretory proteins (Cunha & Young 1991, Donjacour & Cunha 1993). In long-term castrated animals, exogenous androgens initially promote prostatic epithelial proliferation and cytodifferentiation as well as the re-emergence of a smooth muscle stroma. Ultimately, androgen replacement leads to regeneration of a fully differentiated, growth-quiescent gland. These data suggest that the local control of prostatic epithelial proliferation and differentiation occurs through androgenic stimulation of the prostatic stroma, and that the nature of the epithelial response to such a stimulation is predominantly determined by the nature of the stromal cells which are stimulated. Thus, AR-expressing prostatic smooth muscle cells appear to respond to androgenic stimulation by inhibiting epithelial proliferation and maintaining epithelial differentiation, while an AR-expressing fibroblastic stroma (either the urogenital sinus mesenchyme or the stroma from an androgen-deprived adult prostate) may respond to androgens by stimulating epithelial proliferation and eliciting columnar cyto-differentiation.

Genetic changes in human prostatic cancer

It appears that human prostate cancer begins with genetic alteration to the prostatic epithelium. In this regard, a variety of techniques have been useful in demonstrating genetic abnormalities in the prostatic epithelium, both in the primary tumor and at metastatic sites. However, none of these techniques has successfully defined a specific mutation that is characteristic for human prostate cancer. Although no study to date has specifically addressed genetic changes to the prostatic stroma during carcinogenesis, preliminary data from our laboratory using karyotypic analysis and comparative genomic hybridization demonstrate the absence of gross genetic alterations in the stromal cells surrounding a prostate cancer.

The familial concentration of some prostate cancers suggests that risk for this tumor may be inherited in an autosomal dominant fashion. Risk factors for the inherited form of this disease, including early age of onset and multiple affected family members, has allowed for the identification of some putative genetic abnormalities in these patients (Carter *et al.* 1990, 1991, 1992, Smith *et al.* 1996). Loss of heterozygosity has been reported frequently on chromosomes 10q, 7q and 16q in prostate cancer specimens (Isaacs *et al.* 1995). This has resulted in the recent description of a candidate tumor suppressor gene, PTEN/MMAC1, for patients with inherited prostate cancer (Suzuki *et al.* 1998). It must be emphasized, however, that patients with hereditary prostate cancer represent only a minority of prostate cancer patients. Aside from these rare families, prostate cancer appears to be a common but spontaneously arising disease with no consistent pattern of genetic alteration.

Previous studies have described aberrant expression of oncogenes, metastasis suppressor genes and tumor suppressor genes in prostate tumor specimens. While oncogenes such as ras, c-myc, c-sis and neu have been shown to be overexpressed in prostate cancer samples, no single oncogene is consistently overexpressed across a wide range of prostate tumors (Peehl 1993, Wang & Wong 1997). As a result, neither the initiation nor progression of prostate cancer can be linked to a specific oncogene. While experimental overexpression of various oncogenes has demonstrated the potential to initiate and/or promote the disease (Thompson *et al.* 1989, 1993*a*,*b*, Bello *et al.* 1997, Webber *et al.* 1997), the relevance of these interesting findings to clinical disease remains to be established.

A second class of genes which may prove to be important in human prostate cancer progression are the so called 'metastasis suppressor' genes. Examples of such genes include the KAI1 gene and thymosin-beta 15 (a gene which appears to be related to cell motility). KAI1 has been mapped to human chromosome 11p11.2. Introduction of this gene into the metastatic rat AT6.1 prostate cancer cells was shown to suppress prostate cancer metastases (Dong *et al.* 1995). In the human, expression of this gene appears to be reduced in cell lines derived from metastatic prostate tumors (Dong *et al.* 1995, 1996*a*,*b*). Thymosin-beta 15, a 5.3 kDa protein, binds actin monomers, thereby inhibiting actin polymerization (Gold *et al.* 1997). In the rat Dunning tumor model, cell motility has been shown to correlate well with metastatic phenotype. Transfection of these tumor cells with anti-sense thymosin-beta 15 has been shown to cause a decrease in cell motility (Bao *et al.* 1996). Thymosin-beta 15 levels appear to be elevated in human prostate cancer, and levels of this protein have been correlated with Gleason grade (Bao *et al.* 1996). Additional studies have

suggested that there may be other metastasis suppressor genes important in human prostate cancer located on chromosomes 8 and 10 (Ichikawa *et al.* 1996).

The tumor suppressor genes represent a third class of genes that may demonstrate alterations in human prostate cancer specimens. Mutations in the retinoblastoma (Rb) gene have been reported to occur in prostate tumor samples; however, such mutations are present in only a minority of cases (Isaacs 1995, Kubota *et al.* 1995). p53 alterations are also inconsistently found in prostate cancer, appearing in only 20% of cases. In contrast, alterations in E-cadherin may be the most commonly detected defect in human prostate cancers, with up to 50% of cases demonstrating altered expression of this protein.

E-cadherin is a 120 kDa transmembrane glycoprotein which is involved in epithelial cell adhesion (Birchmeier & Behrens 1994, Birchmeier *et al.* 1995, Jiang 1996, Shiozaki *et al.* 1996). The extracellular domain of E-cadherin undergoes calcium-dependent homophilic interactions in adherent junctions and along lateral epithelial membranes. The cytoplasmic domain of E-cadherin is associated with a group of proteins known as catenins that link the intracellular domain of E-cadherin to the actin microfilament network (Jiang 1996). A functional E-cadherin system is required to maintain normal epithelial morphology.

Regulation of E-cadherin is poorly understood. The molecule is developmentally regulated in the embryo where it is initially expressed coincident with the appearance of epithelial tissue at the time of compaction of the embryo. Epithelial to mesenchymal transformation (i.e. the generation of mesoderm through the primitive streak) is associated with loss of E-cadherin, while mesenchymal to epithelial transformation (i.e. formation of the nephron) is associated with increased expression of E-cadherin (Hay & Zuk 1995). A critical role for E-cadherin in development (Takeichi 1988) is underscored by the observation that 'knockout' of the E-cadherin gene in transgenic mice is embryonic lethal (Larue *et al.* 1994). While regulatory elements have been identified in the E-cadherin promoter (Behrens *et al.* 1992), the relevance of these regulatory elements to the biology of E-cadherin remains unclear.

An important role for E-cadherin expression in cellular adhesion is demonstrated by the fact that cells lacking E-cadherin are unable to aggregate or adhere. Furthermore, neutralizing antibodies to E-cadherin cause adherent cells to dissociate. Mutation or deletion of genes encoding E-cadherin or the cadherin-associated proteins (catenins) also render cells non-adherent. Transfection of E-cadherin-negative cells with E-cadherin cDNA leads to reversal of the non-adherent phenotype.

These concepts are of obvious importance to tumor cell invasion and metastasis in the prostate and many other organs. Experimental impairment of E-cadherin with either antibodies or anti-sense cDNA converts non-invasive cells into invasive ones (Vleminckx *et al.* 1991). Moreover, loss of E-cadherin expression has been shown to coincide with the transformation of a well differentiated adenoma into a carcinoma using a transgenic mouse model of pancreatic carcinogenesis (Perl *et al.* 1998). In the prostate, decreased expression of E-cadherin in invasive and metastatic cancers is associated with poor prognosis (Giroldi *et al.* 1994, Umbas *et al.* 1994, Shiozaki *et al.* 1996). While E-cadherin is consistently expressed in normal epithelial cells (including prostatic epithelium) and in various highly differentiated 'non-invasive' carcinoma cells (including prostatic carcinomas), E-cadherin expression is considerably lower (or entirely lacking) in poorly differentiated invasive carcinoma cells (including prostatic carcinomas) (Umbas *et al.* 1992, Giroldi & Schalken 1993, Birchmeier & Behrens 1994, Giroldi *et al.* 1994, Jiang 1996). Bussemakers *et al.* (1993) demonstrated that coincident with the loss of E-cadherin expression, a variant of the rat Dunning prostatic tumor spontaneously changed from a well differentiated, androgen-responsive, slow growing non-metastatic line into an anaplastic, androgen-independent, rapidly growing metastatic line. Similarly, patients with prostatic tumors that do not express E-cadherin, or that have abnormal patterns of E-cadherin expression, appear to have a poorer prognosis than patients with prostatic tumors that express E-cadherin normally (Umbas *et al.* 1994). The relatively common loss of portions of chromosome 16 in human prostatic carcinoma may result in deletion of all or part of the E-cadherin gene which maps to 16q21 (Joos *et al.* 1995). Other mechanisms that may cause dysfunction of cadherin-mediated interactions include increased tyrosine phosphorylation of β-catenin and mutational inactivation of either the extracellular or intracellular domains of the E-cadherin gene (Behrens 1993, 1994). This type of mutational inactivation of the E-cadherin gene has not been described in human prostatic carcinoma but has been reported in endometrial and gastric cancers (Becker *et al.* 1993, Risinger *et al.* 1994). In some high grade prostatic carcinomas, E-cadherin levels may remain relatively normal while α-catenin may be absent (Isaacs *et al.* 1994). The absence of α-catenin also renders the E-cadherin system non-functional (Hulsken *et al.* 1994). For example, impaired E-cadherin function in the PC3 prostatic carcinoma cell line appears to be caused by homozygous deletion of α-catenin (Morton *et al.* 1993)

Previous studies have suggested that 'host factors' may down-regulate E-cadherin expression in carcinoma cells. This observation was made from experiments in which virus-transformed MDCK cells (expressing high levels of E-cadherin and exhibiting an adhesive

In one embodiment, the substrate in the kit is in the form of a probe which is removably insertable into a gas phase ion spectrometer. In another embodiment, the kit further comprises another substrate which can be used together with the substrate comprising the adsorbent to form a probe which is removably insertable into a gas phase ion spectrometer.

In another embodiment, the kit further comprises instructions for suitable operational parameters.

In yet another embodiment, the substrate comprises a hydrophobic group and an anionic group as an adsorbent. In yet another embodiment, the substrate comprises a hydrophobic group as an adsorbent. In yet another embodiment, the substrate comprises a metal chelating group. In yet another embodiment, the substrate comprises a metal chelating group complexed with a metal ion as an adsorbent. In yet another embodiment, the substrate comprises an antibody that specifically binds to a marker, preferably seminal basic protein, as an adsorbent. In yet another embodiment, the washing solution is an aqueous solution.

In yet another embodiment, the kit comprises an antibody that specifically binds to the marker, and a detection reagent. Optionally, the antibody can be immobilized on a solid support.

In yet another embodiment, the kits can further comprise a standard indicating a diagnostic amount of the marker.

While the absolute identity of many markers is not yet known, such knowledge is not necessary to measure them in a patient sample, because they are sufficiently characterized by, *e.g.*, mass and by affinity characteristics. It is noted that molecular weight and binding properties are characteristic properties of these markers and not limitations on means of detection or isolation. Furthermore, using the methods described herein or other methods known in the art, the absolute identity of the markers can be determined.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the amino acid sequence of seminal basic protein.

Figure 2 illustrates a probe comprising spots of adsorbents on the probe surface.

EXHIBIT 2